Experimental Studies on Gallstones in Hamsters

by

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I. INTRODUCTION

Although reports on the pathogenesis of cholelithiasis have been published by many investigators, the mechanism of gallstone formation still remains obscure. In our laboratory, HIKASA[1] in 1960 first pointed out that the formation of gallstone might be due to the deficiency and/or metabolic disturbances of essential fatty acids (EFA), and he and his colaborators demonstrated the various specific effects of EFA on cholesterol metabolism. HIRANO[2], YOSHINAGA[3] and MARUYAMA[4] observed that EFA and pyridoxine have a great influence on the synthesis of bile acids from cholesterol in the liver and their excretion into the bile in rats. SHIODA[5] and TANIMURA[6] have observed that carbohydrate and fat played important roles in the formation of gallstones. In addition, DAM[7], CAIRA[8] and LINDEN[9] have already demonstrated the effects of hormones on gallstone formation. Nevertheless, the etiology of cholelithiasis still remains vague.

In the present studies vitamins, hormones, fatty acids and carbohydrates are discussed as alimentary factors in gallstone formation, and the correlation between them and the development of experimental gallstones is examined in golden hamsters.

II. MATERIALS AND METHODS

Golden hamsters of both sexes weighing 30 to 50 g were used. Until the beginning of the experimental feeding period, the animals received a commercial diet, CE-2 (Central Laboratories of Experimental Animals, Tokyo). They were housed in individual screen-bottomed cages and were weighed weekly. Kneaded synthetic food and water were supplied *ad libitum*. Diets containing fats were replaced every day to prevent oxidation of fatty acids.

These experiments consisted of the following four series :

Series I. Effect of vitamin K_1.

Glucose was used as the carbohydrate component. In each group, ten golden hamsters were fed the vitamin K_1-free diet designed in our laboratory as explained in Table 1. For protein, vitamin free casein (Nutritional Biochemicals Co., OHIO, U. S. A.) was used. Kativ-N (TAKEDA Chemical Industries, Ltd.), as vitamin K_1, was injected intramuscularly in a dose of 2 mg/day. Controls received a glucose fat-free diet with no vitamin K_1.

Table 1. Experimental Diet of Hamsters in Series I.

Group No.	A	B	C	CE-2
Glucose	73.5%	63.5%	63.5%	
Wheat & Corn				ca. 60.0
Fats				3.5
Vitamin-free Casein	20.0	20.0	20.0	24.0
Salt mixture*	5.0	5.0	5.0	6.0
Vatamins**	1.0	1.0	1.0	
Choline chloride	0.5	0.5	0.5	
Sesame oil ^		10.0		
Butter-fat ^ ^			10.0	
Cellulose	—	—	—	4.5

^ Sesame oil : by TAKEMOTO Purified Oil Co.
^ ^ Butter-fat : canned by Snow Brand Milk Products Co.

*Salt Mixture

NaCl	4.6
NaH_2PO_4	9.2
K_2HPO_4	25.3
$CaH_4(PO_4)_2H_2O$	14.3
Ca. lactate	36.9
$MgSO_4$	7.0
KI	2.6
	100.0 g

**Vitamin Mixture (in 100 g diet)

Vitamin	Our Laboratory	CE-2
B_1	1.0mg	0.7mg
B_2	1.5mg	1.0mg
B_6	1.0mg	0.4mg
B_{12}	1 γ	2 γ
Folic acid	0.15mg	0.02mg
Niacin	10.0mg	8.0mg
C	37.5mg	—
Ca. pantothenate	2.5mg	3.0mg
E	1.0mg	1.5mg
A	2,500I.U.	1,000I.U.
D	200I.U.	200I.U.
Choline	(500mg)	140mg

Series II. Effect of various hormones.

Four hormones were used, progesterone (TEIKOKU Zoki Seiyaku Co.), estrogen (Robal by CHUGAI Pharmaceutical Co., Ltd.), thiouracil (TAKEDA Chemical Industries, Ltd.) and cortisone acetate (Cortone by NIPPON-MERK-BANYU Seiyaku Co.). The carbohydrate component in the diet was glucose.

Group A : Progesterone.

Ten hamsters of each sex on the glucose fat-free diet were given intramuscular injections of progesterone, 0.06 mg per g of body weight every other day for 36 days.

Group B : Estrogen.

Ten female hamsters on the glucose fat-free diet were given intramuscular injections of estrogen, 0.01 mg per g of body weight every other day for 36 days.

Group C : Thiouracil.

Eighteen hamsters of each sex received thiouracil in a concentration of 0.02 % by weight in the glucose fat-free diet and received simultaneously at the 0.05 % water solu-

Table 2. Experimental Diets of Hamsters in Series III.

Group	A	B	C
Glucose	63.5%	63.5%	63.5%
Crude casein	20.0	20.0	20.0
Salt mixture	5.0	5.0	5.0
Vitamins	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5
Butter-fat	8.0	9.0	10.0
Linoleic acid*	2.0	1.0	0

* Linoleic acid : 96.5%, by ONO Pharmaceutical Co. Ltd.
Salt mixture and various vitamins are the same as listed in Table 1.

tion for 36 days.

Group D : Cortisone acetate.

Twenty-nine hamsters of each sex on the glucose fat-free diet were given subcutaneous injections of cortisone acetate, 0.04 mg per g of body weight twice a week for 36 days.

Series III. Effects of pure linoleic acid.

Three groups of 10 hamsters each were fed the diets shown in Table 2.

Series IV. Effect of carbohydrate components.

α Starch and β-starch were used as the carbohydrate components. The experimental diets of the hamsters in this series are listed in Table 3.

Table 3. Experimental Diets of Hamsters in Series IV.

Group No.	A	B	C	D	E	F	G	H
α-Starch*	73.5%	53.5%	52.5%	52.5%				
β-Starch**					73.5%	53.5%	52.5%	52.3%
Butter-fat		20.0	20.0	20.0		20.0	20.0	20.0
Crystalline Cholesterol			1.0	1.0			1.0	1.0
Neomycin ^				2 g /L Water-solution				
Cholic acid ^ ^								0.2
Crude casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Salt mixture	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* α-Starch : Instant-Mashed-Potato by Snow Brand Foods Industry Co. Ltd.
** β-Starch : as usually used in other experiments.
^ Neomycin : by ONO Pharmaceutical Co. Ltd.
^ ^ Cholic acid : by NAKARAI Chemicals Co.

After the animals in these four series had been fed for 5 weeks, they were operated on under nembutal anesthesia. The common bile duct was ligated, the gallbladder removed, and a fine polyethylene tube was inserted into the hepatic duct through the cystic duct. The hepatic bile was collected for 24 hours and analyzed as follows :

(1) Biliary cholesterol was measured according to LIEBERMAN-BURCHARD's method modified by MARTENSSON (1963)[10].

(2) Bile acid concentration was calculated by adaptation of the procedure reported by MOSBACH et al. (1954)[11].

(3) Phospholipids in the hepatic bile were extracted by BLOOR's solution and measured by the method of MORRISON (1964)[12].

(4) Total fatty acids were analyzed by gas liquid chromatography by the method of HIKASA et al. (1963)[13].

After cholecystectomy, the gallstones in the gallbladder were collected and washed with distilled water and dried immediately. The stones were classified according to their microscopic appearance and chemical analysis.

III. RESULTS

Series I.

Cholesterol gallstones were completely prevented in hamsters receiving injections of vitamin K_1, those fed on the glucose fat-free diet or the glucose sesame oil diet, while the animals fed on the glucose butter-fat diet developed cholesterol gallstones. The total bile acid concentration in the hepatic bile of animals fed the glucose fat-free diet was higher than in those fed the sesame oil or butter-fat diet. The ratio of total bile acids to cholesterol was much higher in the gallstone negative groups than in those developing gallstones. All the hamsters on the glucose butter-fat diet grew healthily in the early stage of the experiment but towards the end of the 3rd. week of the experiment their fur began look wet and flattened. Under these conditions, the animals fed the glucose butter-fat diet were operated on earlier than the others. The biological analysis of the bile of hamsters in this series is shown in Table 5.

Series II.

Group A.

Of the 10 hamsters in this group, 2 died at the end of the 4th week of the experiment, and all the animals had pure white cholesterol gallstones in their gallbladders. The gallbladders of the majority of animals were filled with deep yellow bile and were distended. However, the gallstones could easily be seen through the gallbladder wall. Generally, the gallstones were just visible to the naked eye, and the largest one measured was about 0.7 mm in diameter; the majority of them were too large to pass through the cystic duct. The concentration of total bile acids and phospholipids in the hepatic bile of animals injected with progesterone was lowest in this series. The ratio of total bile acids to cholesterol (B/C) and the ratio of phospholipis to cholesterol were also lowest in this series (Table 5).

Group B.

Of the 8 female hamsters receiving estrogen, 4 developed cholesterol gallstones. The stones were similar to those in group A.

Group C.

Of the 11 hamsters receiving thiouracil, 3 developed cholesterol gallstones. One of those stones stayed in the cystic duct.

Group D.

Of the 29 hamsters receiving cortisone, 9 died one day before the operation; 6 developed cholesterol gallstones; 6 developed pigmented gallstones; and 17 animals had no gallstones.

Series III.

Table 4. Incidence of Gallstones in Hamsters fed Various Diets.

Experimental series & groups		Diet characteristics	No. of animals	Initial body weight (g)	Weight gain during 4 weeks (g)	Maximal feeding period (days)	Survivors after 5 weeks	Incidence of gallstones among survivors	
								Cholesterol stones (%)	Pigmented stones (%)
I	A	Fat-free	10	39.7	0.3	36	10	0	0
	B	10% Sesame oil	9	40.0	4.2	36	8	0	11
	C	10% Buffer-fat	8	39.5	−3.4	30	8	50	13
Control		Glucose, Fat-free	10	51.5	−1.1	35	7	86	0
II	A	Progesterone	10	33.2	10.2	36	8	100	0
	B	Estrogen	10	38.9	1.1	36	8	50	0
	C	Thiouracil	18	40.4	0.9	36	11	27	0
	D	Cortisone	29	35.8	4.3	36	20	21	21
III	A	Butter-fat : Linoleic acid 8 . 2	10	52.4	5.2	30	8	20	60
	B	9 : 1	10	43.9	−1.8	30	7	30	20
	C	10 : 0	10	49.1	5.5	43	8	75	0
IV	A	α-Starch, Fat-free	10	42.5	31.2	36	6	0	10
	B	α-Starch, 20% Butter-fat	23	48.7	50.3	36	20	57	21
	C	α-Starch, 20% Butter-fat, 1% Cholesterol	10	45.5	46.5	36	10	50	50
	D	α-Starch, 20% Butter-fat, 1% Cholesterol, Neomycin	10	41.6	41.7	36	8	0	100
	E	β-Starch, Fat-free	10	37.5	3.0	36	8	0	0
	F	β-Starch, 20% Butter-fat	15	37.5	29.4	36	12	0	13
	G	β-Starch, 20% Butter-fat, 1% Cholesterol	10	37.6	29.6	36	6	20	60
	H	β-Starch, 20% Butter-fat, 1% Cholesterol, 0.2% Cholic acid	10	44.7	7.7	36	7	10	10

Group A.

All the animals grew healthily during the initial stage of the experiment, but after about 3 weeks they became rather weak. Therefore, they were operated on immediately. The administration of pure linoleic acid in place of 20 % butter-fat in the diet greatly prevented the formation of cholesterol gallstones, and favoured that of pigmented gallstones. The pigmented gallstones produced by animals in group A were almost black and majority of them were very hard. The concentration of total bile acids was the highest in this series and the ratio of total bile acids to cholesterol (B/C) was also the highest.

Group B.

Table 5. Biological Analysis of the Bile of Hamsters fed Various Diets.

Experimental series & groups		Diet characteristics	Cholesterol (mg/dl)	Total bile acids (mg/dl)	Phospholipids (mg/dl)	Ratio of total bile acids to cholesterol	Ratio of phospholipids to cholesterol
I	A	Fat-free	6.8±2.3	72.4±8.4	19.6±6.9	10.6±0.7	2.9±0.7
	B	10% Sesame oil	4.9±0.9	48.5±8.8	20.8±8.1	9.7±0.4	4.2±0.5
	C	10% Butter-fat	7.5±1.4	36.5±1.7	10.1±2.2	4.9±0.3	1.3±0.1
Control		Glucose, Fat-free	10.8±1.4	56.3±7.1	17.9±7.8	5.3±1.2	1.6±0.4
II	A	Progesterone	9.1±3.5	26.7±1.8	9.2±1.4	2.9±0.8	1.0±0.3
	B	Estrogen	9.1±1.3	30.9±1.7	11.9±2.3	3.4±0.5	1.3±0.3
	C	Thiouracil	7.8±4.3	45.2±2.0	20.2±5.8	5.8±1.0	2.6±0.4
	D	Cortisone	7.7±2.4	46.9±8.7	81.2±14.3	6.1±1.4	10.5±3.2
III	A	Butter-fat : Linoleic acid 8 : 2	9.0±1.3	107.8±2.8	41.3±10.0	11.9±0.9	4.6±0.7
	B	9 : 1	10.7±1.7	87.7±1.9	42.4±9.8	8.2±0.3	4.0±0.6
	C	10 : 0	12.9±2.5	56.8±14.5	13.7±7.5	5.8±1.6	1.0±0.6
IV	A	α-Starch, Fat-free	6.3±2.8	147.4±12.5	73.5±5.4	23.4±0.7	11.7±0.3
	B	α-Starch, 20% Butter-fat	7.3±1.4	43.4±5.1	64.5±1.4	5.9±0.8	8.8±0.5
	C	α-Starch, 20% Butter-fat, 1% Cholesterol	7.0±1.5	46.9±1.6	35.8±11.1	6.7±0.2	5.1±0.3
	D	α-Starch, 20% Butter-fat, 1% Cholesterol, Neomycin	10.1±1.9	142.0±6.0	94.3±3.0	14.1±0.2	9.3±0.6
	E	β-Starch, Fat-free	6.0±0.7	79.3±10.1	10.6±3.1	12.0±2.8	1.6±0.4
	F	β-Starch, 20% Butter-fat	—	—	—	—	—
	G	β-Starch, 20% Butter-fat, 1% Cholesterol	4.0±0.7	40.7±2.6	23.5±5.0	10.2±0.1	5.9±0.1
	H	β-Starch, 20% Butter-fat, 1% Cholesterol, 0.2% Cholic acid	10.9±1.3	127.5±7.4	132.2±3.8	11.7±0.3	12.1±0.3

The general condition of the animals was similar to that of those in group A, but the loss of body weight during the last stage of the experiment was much greater. The cholesterol gallstones produced in this group were somewhat larger than in group A and the pigmented gallstones were similar to those in group A.

Group C.

The animals fed the glucose butter-fat diet grew better than the other animals in series III, and many developed cholesterol gallstones. No pigmented gallstones were formed. The concentration of total bile acids and the ratio of total bile acids to cholesterol (B/C) were lower than in the other groups in this series.

Series IV.

All the animals fed α-starch as the carbohydrate component grew well for the 4 weeks of the experiment, but at the end of the experiment they appeared to be rather weak.

Group A.

These were completely free of cholesterol gallstones.

Group B.

The animals fed butter-fat grew better than the other groups and produced many cholesterol gallstones. The body-fur and the epidermal and mucosal surfaces did not show any of the signe of deficiency noted by DAM and CHRISTENSEN[14]. The gallbladders in the majority of these animals were filled with slightly green, yellow bile, and the gallstones could easily be seen through the walls of the gallbladder and cystic duct. The gallstones were generally just visible to the naked eye, and the largest measured about 0.4 mm in diameter (smaller than in group A of series II) and all of them were able to pass through the cystic duct. The cholesterol gallstones were softer than in any other series and could easily be crushed by a forceps. The total bile acid level in the hepatic bile was lowest in this series, and the ratio of total bile acids to cholesterol (B/C) was also lowest.

Group C.

The appearance of the animals and the composition of the hepatic bile were similar to those of group A. Four animals had both cholesterol- and pigmented-gallstones simultaneously; one had cholesterol gallstones and another had pigmented gallstones, while 4 animals had no gallstones at all.

Group D.

The administration of neomycin orally in a 0.2 % water solution did not favour the formation of cholesterol gallstones, but only that of pigmented-gallstones. The total bile acid level in the hepatic bile was highest in this group and the ratio of total bile acids to cholesterol (B/C) was also highest.

Group E.

The animals fed the β-starch fat-free diet gained weight slowly, but had no gallstones.

Group F.

Of the 15 hamsters fed the β-starch butter-fat diet, 2 animals produced pigmented gallstones.

Group G.

Of the 10 hamsters fed the β-starch butter-fat diet plus crystalline cholesterol, 2 animals produced cholesterol gallstones. These stones were so soft that they could be crushed easily by a forceps. The cholesterol level in the hepatic bile was the lowest in this series.

Group H.

The administration of cholic acid did not enhance the growth of the animals. The level of phospholipids in the hepatic bile was the highest in this series.

IV. DISCUSSION

Gallstones have been produced experimentally in dogs[15], rabbits[16][17][18][19][20], guinea pigs[21][22], hamsters[6][23][24], rats[25] and mice[26] by dietary means. However, only in hamsters can they be produced by cholesterol-free diets. In hamsters, disturbance of cholesterol metabolism occurs more easily than in other experimental animals[27]. In the present studies, hamsters were used, because they are rodents, and albino rats had been tested previously in our laboratory.

DAM and CHRISTENSEN[28] first reported the alimentary production of gallstones in hamsters in 1952, and FORTNER[29] (1954), CAIRA et al.[8] (1958), LINDEN[30] (1959), DREW[31] (1963), WATANABE[32] (1964), SHIODA[5] (1965) and TANIMURA[6] (1965) have confirmed their findings. However, with the exception of CAIRA, they did not attempt to give the animals vitamin K, and CAIRA did not give them natural vitamin K_1.

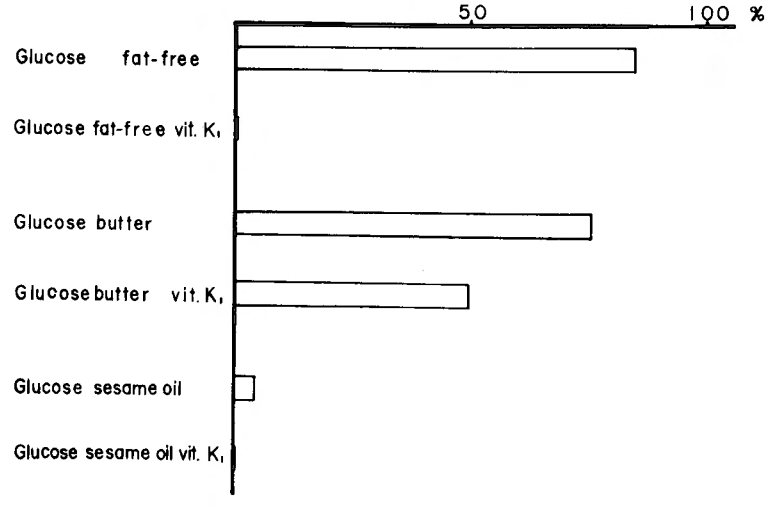
In hamsters fed glucose or sucrose fat-free diets, the intestinal flora did not increase and they occasionally developed diarrhea[33].

Injections of vitamin K_1 prevented cholesterol gallstones in hamsters fed the glucose fat-free diet, but not in those fed the glucose butter-fat diet. In animals on the glucose fat-free diet, the total bile acid concentration in the hepatic bile was increased by the administration of vitamin K_1, but no significant changes were observed in the bile of animals on the glucose butter-fat diet. It is now well known that vitamin K_1 is closely involved in the formation of dehydrogenases[34][35][36]. Electron microscopy has shown that vitamin K_1 has a reparative function in the damaged mitochondria of liver cells[37]. It is supposed that vitamin K_1 increases the excretion of bile acid by the liver cells.

Vitamin K_1 appears to be essential in maintaining cholesterol biosynthesis in the normal liver[34] and its deficiency probably increases the biosynthesis of cholesterol in the liver. Why were cholesterol gallstones more apt to develop on a glucose than on a starch diet? The explanation seems to be that the glucose diet causes a shift of the intestinal flora, *dysbacteria*, because of insufficient roughage and consequently deficiencies of many vitamins, notably natural vitamin K (especially vitamin K_1). When the extraordinary increase of cholesterol biosynthesis and the disturbed catabolic process of cholesterol in the liver occur at the same time, the excess cholesterol excreted in the bile begins to crystallize and precipitates when the total bile acid concentration falls. As a result, cholesterol stones are formed. Moreover, it is well known that the gallbladder is necessary for cholesterol gallstone formation.

Fig. 1

Effect of Vitamin K_1 on the Formation of Gallstones.

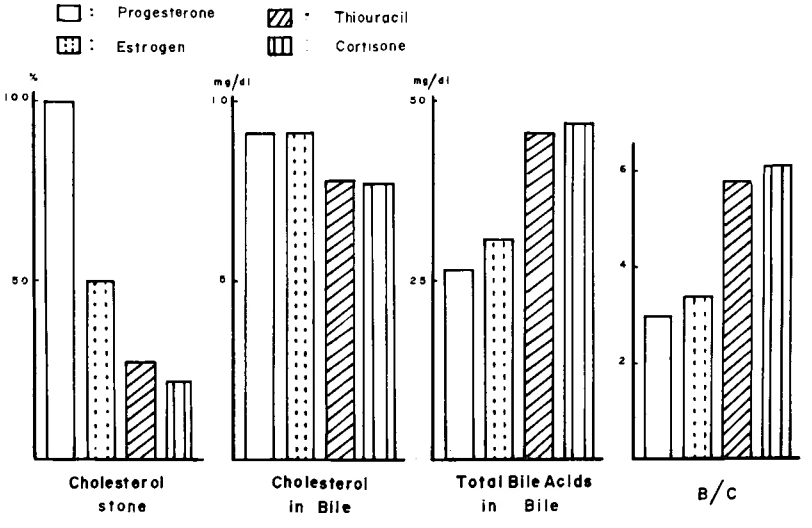


In human beings the frequent observation of cholelithiasis during pregnancy, in patients with diabetes mellitus and in middle aged obese females suggests a metabolic disturbance of female sex hormones. CAIRA[8] stated that the administration of progesterone or estrogen decreased the production of gallstones in hamsters fed a sucrose fat-free diet. DAM[7] has recently reported that treatment of male hamsters with testosterone and of female hamsters with estradiol had no influence on gallstone production, but that treatment with progesterone decreased the incidence of cholesterol stones. However, they used sucrose as the carbohydrate component. Glucose was used in our laboratory instead of sucrose, because the occurrence of cholesterol gallstones is not uniforme in hamsters fed on a sucrose diet. SHIMURA[38] observed no alternation of the bile components in pregnant goats.

The administration of progesterone to the animals in series II showed no influence of cholesterol gallstone prevention, but the administration of estrogen decreased the incidence of gallstones. The effect of estrogen on cholesterol metabolism in the liver might explain this finding, but this inhibitory action is not sufficient, because the effect of estrogen on the liver is not direct. BOCKUS et al[39] found a higher incidence of hypothyroidism in patients with cholelithiasis than expected, but it has not been shown that gallstones are more common in hypothyroidism or myxedema. Indeed, YUGENBOURG and SHALEPAKOFF[40] reported the occurrence of cholelithiasis in 27 of 280 patients with thyrotoxicosis. It was observed that thyroxin treatment increased the excretion of cholic acid into the bile of rats, and thiouracil had no influence on bile components[41][42][43]. LINDEN[9] stated that d-thyroxin decreased the incidence of cholesterol gallstones in animals fed a sucrose diet. The administration of thiouracil in this study, however, partially reduced the production of cholesterol gallstones, but not completely. Thyroxin accelerates cholesterol biosynthesis in the liver, but thiouracil has a competitive effect on cholesterol biosynthesis in the liver, and cholesterol in the liver is lowered in animals receiving thiouracil.

Fig. 2

Effect of Various Hormones on Bile Composition in Hameters.



MURAOKA[44], in our laboratory, has stated that arachidonic acid plays an important role in the formation of glucocorticoid hormone in the adrenal cortices of the rat. FUKUDA[45] observed that the serum tetraenoic acid level in patients with cholesterol stones was low, and that their adrenocortical function decreased when ACTH Z was injected. The administration of glucocorticoids partially inhibited cholesterol gallstone formation, but it produced amorphous pigmented stones.

Thus, these hormones have some inhibitor effect on gallstone formation, but carbohydrates and fats seem to play a greater role.

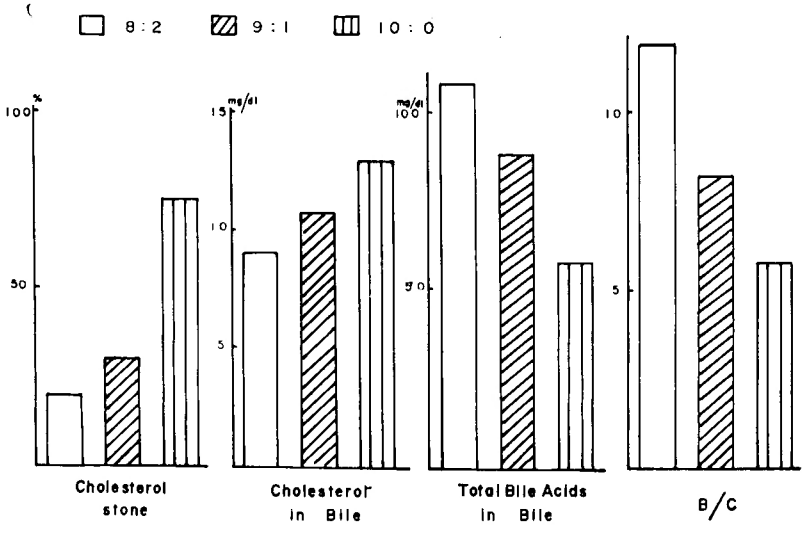
TANIMURA[6] found that animals fed on a glucose butter-fat diet produced cholesterol gallstones regularly and stated that the higher the ratio of EFA to saturated fatty acids plus oleic acid, the lower the incidence of cholesterol gallstones. The administration of pure linoleic acid in place of 10 % or 20 % butter-fat greatly decreased the incidence of cholesterol gallstones. As complete inhibition was not observed, linoleic acid *per se* was not able to prevent cholesterol gallstone formation; this fact suggests that the biosynthesis of cholesterol in the liver is increased in animals with cholesterol gallstones. MUROYA[46] demonstrated that the biosynthesis of cholesterol from 2-C^{14} acetate in the liver was increased more in animals fed a glucose fat-free diet than in those fed a starch fat-free diet.

SHIODA[5] and DAM et al[47] observed great differences of cholesterol gallstone formation between the glucose fat-free diet group and the starch fat-free diet group. This difference was due to the alteration of bile components, i. e. an increase of cholesterol and a decrease of bile acids. TANIMURA[6] stated that the phospholipid and fatty acid constituents did not show any difference between these two groups.

HIKASA[48] pointed out that alteration of the intestinal flora (dysbacteria) played the greatest role in the formation of gallstones, and it was also observed in this study that the feeding of easily digestible carbohydrates resulted in a high incidence of cholesterol galls-

Fig. 3

Effect of Pure Linoleic Acid on Bile Composition in Hamsters.



tones. TANIMURA[49] observed that in animals fed starch digested by some enzymes, the incidence of cholesterol gallstones was as high as that in animals on a glucose fat-free diet. As is generally known, untreated raw starch, such as potato starch, is not soluble in cold water and is not degraded by any digestive enzyme. It is, therefore, of the β-form. In our laboratory potato starch (β-starch) was used as a routine source of carbohydrate. But, when this β-form starch is heated in water to a certain temperature (i. e. cooked), its chemical micells suddenly separate, swell, and become gelatinized to form α-form starch which is easily digested and absorbed. Mashed potato is this α-form starch, and human beings usually take carbohydrate in the form of α-starch.

However, animals fed α-starch as the source of carbohydrate did not develope cholesterol gallstones. The administration of large amounts of lower saturated fatty acids induced a high incidence of cholesterol gallstones in animals fed a α-starch diet.

The chemical composition of these cholesterol stones was very similar to that produced in the glucose fat-free or butter-fat diet group. However, the administration of 1% crystalline cholesterol caused almost the same incidence of cholesterol stones (group C). Cholesterol is stored in the livers of hamsters when given exogenously and the hepatic cholesterol level in this group was much higher (above 10 times) than in the other groups.

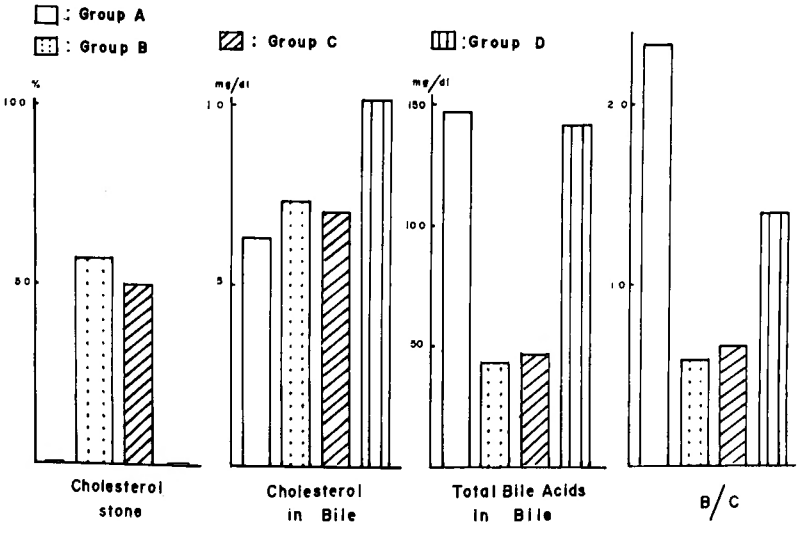
It was observed that neomycin had an anti-cholesterolemic effect in human beings[50][51] presumably because of the increased conversion of cholesterol to bile acids.

The administration of neomycin to hamsters fed the α-starch butter-fat diet prevented the occurrence of cholesterol stones, but pigmented stones were observed in all the experimental animals. These pigmented stones contained large amounts of bile salts (TANIMURA[6], DREW[27] and PRANGE[52]) believed to have precipitated by the alteration of the enterohepatic bile acid circulation following the administration of neomycin.

LINDSTEDT and NORMAN[53] observed that the half life of C^{14}-cholic acid was leng-

Fig. 4

Effect of α-Starch as the Carbohydrate Component on Bile Composition in Hamsters.



thened by the administration of antibiotics. However, neomycin seemed to have a different effect on the liver cells than could be accounted for simply by the change in intestinal flora.

The administration of lower saturated fatty-acids did not produced cholesterol stones in animals fed the β-starch diet (group F).

CALDWELL et al.[54] obtained cholesterol stones in mice by feeding 1 % cholesterol and 0.5 % cholic acid.

The administration of crystalline cholesterol and cholic acid in this study produced a cholesterol stone in only one male hamster and this stone was very small (group H). The administration of crystalline cholesterol alone had a similar result (group F). These stones (in group F and H) showed some differences from those produced in animals fed a glucose fat-free diet.

In a word, the results in series IV show that the α-starch diet group produced many more stones than the β-starch group and that large amounts of lower saturated fatty acids must be given to animals at the same time.

In our opinion, these findings definitely prove that the metabolic disturbance of EFA due to large amounts of lower saturated fatty acids is the important factor in cholesterol gallstone formation. Moreover, they agree with the statistical findings in human beings with cholesterol stones that cholesterol gallstones are more frequent among Europeans and Americans, who consume more butter-fat and other animal-fat containing a greater amount of lower saturated fatty acids.

V. SUMMARY AND CONCLUSION

Young golden hamsters were fed on various diets, and were injected with various substances for 4～5 weeks in this investigation of the relationship of several factors, such as vitamins, hormones, linoleic acid and α-starch, to the incidence of experimental gallstone production in hamsters.

(1) Cholesterol gallstones were prevented completely in animals on a glucose fat-free diet by the administration of vitamin K_1.

(2) The administration of progesterone had no influence on the prevention of cholesterol gallstone formation.

(3) The administration of estrogen to the glucose fat-free diet group showed a certain degree of protection from cholesterol gallstone formation.

(4) Oral thiouracil also prevented the formation of cholesterol gallstones to some extent.

(5) The administration of cortisone prevented cholesterol gallstone formation more than did other hormones, and favoured the formation of amorphous pigmented stones.

(6) The addition of pure linoleic acid to the glucose butter-fat diet had some preventive effect on cholesterol gallstone formation.

(7) Animals fed on an α-starch fat-free diet did not develop cholesterol gallstones, but there was a high incidence of cholesterol gallstone formation in animals fed excessive amounts of lower saturated fatty acids, even when *starch* was the main source of carbohydrate.

(8) Animals fed β-starch butter-fat (lower saturated fatty acids) and crystalline

cholesterol occasionally developed cholesterol gallstones.

(9) The addition of neomycin to the α-starch butter-fat diet protected hamsters from cholesterol gallstone formation completely, but they developed amorphous pigmented stones.

(10) The present studies show that an α-starch diet favours cholesterol gallstone formation, since it is easily digested and absorbed and that to produce them large amounts of lower saturaetd fatty acids must be given to the animals at the same time.

Thus, it is to be expected that cholestrol gallstones form more frequently in Europeans and Americans because they consume more butter-fat and more α-starch.

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和文抄録

ハムスターを以てする胆石，就中コレステロール系結石の成因についての実験的研究

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胆石の成因については，従来数多くの学説が唱えられて来た．ということは，まだ決定的な学説の画立されていないことを物語るもので，その真の成因はまだ不明のまま貽されているものといつても過言でない．

われわれはコレステロール系結石なるものが，従来本邦人よりも欧米人に，本邦人にあつても田舎の人よりも都会人に，また戦後食餌の質的変遷に伴つて本邦に於ても欧米のそれが発生率に近ずきつつある点に鑑み，特にコレステロール系結石形成の原因として食餌性因子の存在を重視，かかる観点からハムスターを用いて専らコレステロール系結石の成因を実験的に追究して，次のような結論に到達した．

(1) 既に教室に於て行なわれて来たこの方面の研究によつて，われわれはコレステロール系結石の形成が肝におけるコレステロールの合成と分解のアンバランスによつて招来されるもので，胆嚢は結石形成の場を提供していることを明らかにすると共に，上述のような肝におけるコレステロールの合成と分解のアンバランスを来たす理由の一つとして腸内細菌叢の所謂Dysbacteria なる現象を重視して来た．そして，かかる状態に陥つた個体では，腸内細菌叢によつて合成されるビリドキザール燐酸とビタミンK_1の欠乏状態が惹起されていることが当然考えられた．

(2)このような Dysbacteria なる現象は，上部腸管で吸収される Glucose あるいは Sucrose といつた糖質をその糖質補給源とした無脂質食餌を投与すれば，当然そこに現出され，確かにコレステロール系結石を胆嚢内に高率に発生せしめ得る．しかし，そのような際に体内に当然欠乏しているものと考えられるビタミンK_1を投与すると，予想通りコレステロール系結石の形成は完全に抑制された．なお，ビリドキザール燐酸と不可欠脂酸の併用投与がコレステロール系結石の形成を抑制することは協同研究者谷村の既に報告したところである．

(3) しかし，日常人体は糖質の全てを Glucose, Sucrose といつた型で摂取しているものではない．常に生

の澱粉，即ちβ-澱粉に適量の水分を添加，消化吸収の良好なα-澱粉の型にいつたん変じて摂取しているわけであるから，上述の事実を以て，直ちに人体のコレステロール系結石の成因として云々することは許されない．また，コレステロール系結石患者と雖ども不可欠脂酸は平素から充分に摂取しており，その肝臓中のリノール酸の欠乏はみられない．従つて，糖質補給源としてα-澱粉を投与，かつ脂質をも充分に投与した状態下でもコレステロール系結石が実験的に作製されなければ，真のコレステロール系結石の成因が解明され得たものとはいい難い．

(4) そこで，まずα-澱粉を糖質補給源とした無脂質食餌をハムスターに投与してみたが，それのみではコレステロール系結石を実験的に作製することは不可能であつた．

(5) 次いで，体内にピリドキザール燐酸やビタミン K_1 がある程度存在し乍らも，結果的には Dysbacteria と同じ状態を現出するに至るものと思われる各種条件を検討すると共に，教室に於て既に行なわれた業績をもとに，飽和酸就中低級飽和酸を比較的豊富に含有するバター脂を糖質補給源としてα-澱粉を使用した無脂質食に添加，それをハムスターに投与することによつて，高率にコレステロール系結石を澱粉を投与しながらも実験的に作製することに成功した．

(6) しかし，バター脂を添加した食餌であつても，α-澱粉より消化の不良な，換言すれば腸内細菌の栄養源としては却つて好都合と思われるβ-澱粉に置換すると，コレステロール系結石は再び実験的に作製し得なくなる．

(7) 従つて，コレステロール系結石なるものは，飽和酸就中低級飽和酸を豊富に含有する動物性脂質が大量に摂取された際に形成され得るものであるが，また同時に摂取する糖質の質的，量的問題がその形成に大きな影響を及ぼしていることが判明した．

(8) 故に，人体におけるコレステロール系結石は，動物性脂質が大量に摂取され，それに伴つて糖質摂取量が相対的に減じかつそれが消化良好な糖質のみからなる場合に形成され得るものと考えられる．それに反して，動物性脂質の摂取量の小なる場合，糖質摂取量が大なる場合は勿論のこと，仮令糖質摂取量が相対的に小なる場合でも，それがセルローズ，β-澱粉といつた比較的消化され難い糖質を含有しているような際にも，コレステロール系結石は形成され難くなるものと思われる．

(9) 外因性のコレステロールの大量摂取は却つてコレステロール系結石の形成を抑制した．ということは，コレステロール系結石の形成に当つては，肝におけるコレステロールの合成が異常に亢進していることの必要なことを示唆するものであろう．

(10) ホルモン剤の投与による実験的コレステロール系結石の形成に及ぼす影響も観察したが，それらホルモンの中では，コーチゾンが最もコレステロール系結石の形成を強く抑制した．而して，コレステロール系結石の形成にピリドキザール燐酸の活性低下を介する不可欠脂酸の体内代謝障害にもとずいて二次的に招来される副腎皮質機能の低下もまたコレステロール系結石の形成に対して促進的に作用することが示唆され得た．